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Microbial food webs in hypertrophic fishponds: Omnivorous ciliate taxa are major protistan bacterivores

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Abstract

Despite the importance of shallow lakes worldwide, knowledge of microbial components, the base of their food webs, remains scarce. To close this gap, we investigated planktonic microbial food webs, in particular protistan bacterivory (for both ciliates and heterotrophic nanoflagellates [HNF]), in 10 shallow hypertrophic fishponds in South Bohemia (Czech Republic). We used fluorescently labeled bacteria as bacterivory tracers to estimate how abundant protistan populations in fishponds ($4\text{--}25 \times 10^3$ HNF mL⁻¹ and $55\text{--}770$ ciliates mL⁻¹) contribute to total bacterial mortality. Fluorescence microscopy, innovative image processing tools, and quantitative protargol staining were combined to detect major bacterivorous and omnivorous ciliate taxa. We quantified bacterial production, bacterivory by individual ciliate species, total ciliates, and total protistan bacterivory in all fishponds. On average, ciliate bacterivory was comparable to that of HNF, accounting for 56% and 44% of total protistan grazing, respectively. We found that primarily bacterivorous Peritrichia (genera *Vorticella*, *Epistylis*) and Scuticociliata (*Cyclidium* spp.) contributed only moderately (mean 26%) to total ciliate bacterivory. Unexpectedly, but highly abundant omnivorous *Halteria/Pelagohalteria* (Stichotrichia) and, to a lesser extent, also omnivorous *Rimostrombidium* spp. (Oligotrichia) contributed significantly more (mean 71%) to total ciliate bacterivory than typical bacterivorous taxa. This suggests that unselective grazers, which feed on a broader size spectrum from bacteria to small algae, may have a considerable competitive advantage in hypertrophic environments rich in small particles. Moreover, a meta-analysis of available literature data supports our hypothesis that the role of ciliate bacterivory increases significantly, relative to HNF bacterivory, along a trophic gradient toward hypertrophic habitats.

The importance of the microbial food web from the level of bacteria and smallest phytoplankton through to protists, as a major trophic resource food base for zooplankton, is widely recognized (Jack and Gilbert 1997; Jürgens and Jeppesen 2000; Sommer et al. 2012). The seasonal succession of plankton in freshwater systems is conceptually described in the Plankton Ecology Group (PEG) model that currently also summarizes the

knowledge on possible major pathways through which protists shape food web dynamics (Sommer et al. 2012). We also have a solid theoretical base for evaluating feeding preferences and functional responses of distinct pelagic protistan groups to various food resources (e.g., Montagnes et al. 2008). However, most of our knowledge is derived from studies of cultured protistan species (Wickham 1995; Weisse et al. 2016) and there is relatively little empirical evidence or direct quantification of the contribution of different protistan groups to in situ carbon flows (e.g., Sanders et al. 1989; Gaedke et al. 2002; Posch et al. 2015; Šimek et al. 2018). We also lack knowledge concerning how microbial food webs may vary in different systems.

Compared to meso- and eutrophic systems, considerably little is known about food webs in hypertrophic shallow lakes. Such systems are not only abundant but can harbor a huge diversity of rapidly growing microbes, with unique and highly complex trophic interactions (Sommaruga and Robarts 1997). In hypertrophic

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systems with high phytoplankton biomass and productivity (Sommaruga and Robarts 1997; Comerma et al. 2003), planktonic ciliate densities can reach hundreds to thousands per milliliter (e.g., Nakano et al. 1998; Šimek et al. 2000). However, we lack information on their trophic role. Although heterotrophic nanoflagellates (HNF) are generally considered the major bacterivores in most aquatic systems (Berninger et al. 1991; Arndt et al. 2000; Boenigk and Arndt 2002), there are indications that the role of ciliates as bacterivores may increase along the trophic gradient (Beaver and Crisman 1989; Nakano et al. 1998; Šimek et al. 2000). This increase in ciliate bacterivory seems to be connected with changes in ciliate assemblage composition and prevailing feeding modes. The omnivorous genera *Halteria*, *Pelagohalteria*, *Strombidium*, and *Rimostrombidium* often numerically dominate freshwater pelagic ciliate assemblages in meso-eutrophic systems (Sanders et al. 1989; Macek et al. 1996; Šimek et al. 2000 and references therein). Šimek et al. (2000) suggested that these small fine-filter feeding omnivorous species might be important pelagic bacterivores in environments with high concentration of both picoplankton ($< 2 \mu\text{m}$) and nanoplankton ($2\text{--}20 \mu\text{m}$, dominated by small algae) since they exploit a wider size range of food items compared to specialized bacterivorous ciliates. Thus, the original view of a minor role of ciliate bacterivory proposed by Fenchel (1980) for pelagic systems may require some revision.

Hypertrophic fishponds are ideal sites for examining ciliate assemblage composition and bacterivory rates of individual forms. Since these hypertrophic systems are extremely rich in small food particles, such as free-living and aggregated bacteria, detrital flocks, autotrophic picoplankton, small algae, and HNF (Sommaruga and Robarts 1997), bacterivory and omnivory are presumably the principal feeding strategies of major ciliate taxa there. For our study, we chose 10 fishponds, representing a broad variety of hypertrophic study sites. We used a combination of techniques to detect the major ciliate bacterivores and their prevailing feeding modes. To identify taxa, we used the classical protargol staining technique. To assess ciliate bacterivory, tracer methods with fluorescently labeled bacteria (FLB) were employed to examine species-specific bacterivory rates, and semi-automated image processing to visualize ingested prey with different optical properties (labeled FLB vs. autofluorescing phytoplankton cells). We also estimated bacterivory by HNF using the FLB technique.

The aim of our study was to examine the role of ciliates as bacterivores in hypertrophic aquatic systems with two major hypotheses: (1) Small omnivorous ciliate taxa, rather than specialized, primarily bacterivorous ciliates (such as scuticociliates), are major bacterivores in hypertrophic habitats. (2) There is a general trend of increasing ciliate bacterivory relative to HNF bacterivory along the trophic gradient toward hypertrophic systems.

Materials and methods

Study sites and field sampling

Ten artificial, nutrient-rich, shallow ponds in South Bohemia, Czech Republic (for details, see Supporting Information Tables S1,

S2), used for fish production, were selected as study sites of microbial food web interactions. All ponds are polymictic, eutrophic to hypertrophic waterbodies (area $0.02\text{--}2.28 \text{ km}^2$) with an artificially controlled fish stock. Their sampling covered the growth season from early April to late September 2017. Two main localities (Dehtár and Rod) were sampled monthly; six other ponds were sampled three times (April, June, and August) and two ponds twice (June and August) during the season (see Supporting Information Table S1). Thus, the plankton sample from each sampling date represents an independent observation.

Mixed water samples from the surface layer were taken by a van Dorn sampler (length of 1 m, 6.4 L volume) and filled into a washed 50-liter plastic container from seven sampling points along a linear transect of the open water zone of the pond, thus producing a horizontally integrated water sample. All subsamples for plankton and chemical analysis were taken from this integrated water sample (a volume of 45 L) as follows: Two subsamples were filtered through a $200\text{-}\mu\text{m}$ mesh into a sterile glass bottle for live microbial analyses ($\sim 2 \text{ L}$) and into a plastic bottle for chemistry and seston analyses (ca. $3\text{--}5 \text{ L}$); both subsamples were transported to laboratory within 2 h. Three other unfiltered subsamples were taken for microbial analyses on fixed samples: 100 mL for phytoplankton and 100 mL for ciliates both preserved with Lugol's solution (50 mL were postfixed with Bouin's fluid for analyses of ciliate assemblages), and 120 mL preserved with formaldehyde (2% final concentration) for further analysis of abundances of bacteria, picocyanobacteria, and HNF.

Crustacean zooplankton were concentrated by filtering a total volume of 30 L of the remaining mixed sample through a $200\text{-}\mu\text{m}$ plankton net into another clean container. In the last step, rotifers were concentrated from 5 or 15 L of this filtrate by filtering through a $20\text{-}\mu\text{m}$ mesh, which resulted in the size fraction of $20\text{--}200 \mu\text{m}$. Rotifers were preserved by formaldehyde (4% final concentration). Crustacean samples were gently preserved in a mixed sugar-formaldehyde solution at a final concentration of 4.5% (wt/vol) of formaldehyde.

Bacterial and picocyanobacterial abundance and sizing of bacterial cells

Duplicate 0.4- to 1-mL formaldehyde-preserved subsamples for both bacteria and picocyanobacteria were filtered onto black $0.2\text{-}\mu\text{m}$ pore-size filters (Osmonics, Livermore, CA), stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), and enumerated by epifluorescence microscopy (Olympus BX 53; Optical, Tokyo, Japan). Picocyanobacteria were enumerated using chlorophyll autofluorescence. To estimate bacterial mean cell volume (MCV), cells (> 300) were sized by using a semiautomatic image analysis system (NIS-Elements 3.0, Laboratory Imaging, Prague, Czech Republic; Šimek et al. 2014).

Bacterial production

Bacterial production (BP) was measured via the thymidine incorporation assay. Triplicate 5-mL subsamples were incubated for 30 min at in situ temperature with 20 nmol L^{-1} [methyl- ^3H]

thymidine (Moravek, Brea, California, U.S.A.), then preserved with neutral buffered formaldehyde (final concentration 2% wt/vol), filtered through polycarbonate membrane filters (Osmonics) of 0.2- and 1- μm porosity, and extracted 10 times by 1 mL of ice-cold 5% trichloroacetic acid. Radioactivity on filters was measured with a Packard TriCarb (Packard, Ramsey, U.S.A.) scintillation spectrometer and corrected for blanks prefixed with 2% formaldehyde processed in parallel. Thymidine incorporation into the free bacteria (the size fraction 0.2–1 μm) was calculated as the difference between the radioactivity retained on 0.2- and 1- μm filters. Cell production rates were calculated using the empirical conversion factor of 2×10^{18} bacterial cells mol^{-1} thymidine (Bell 1990; Ducklow and Carlson 1992).

Tracer technique to estimate flagellate and ciliate bacterivory

Flagellate and ciliate bacterivory rates were estimated using FLB (Sherr and Sherr 1987) prepared from a mixture of isolated strains from the genus *Limnohabitans* and *Polynucleobacter*. The strains are typical and highly abundant members of bacterioplankton in lakes (Jezbera et al. 2012) and were isolated from Římov reservoir: *Limnohabitans planktonicus* (short rods, MCV of 0.135 μm^3) and *Limnohabitans parvus* (short rods, MCV of 0.055 μm^3 , see Kasalický et al. 2013) and one undescribed strain (czRimov8-C6) was from the PnecC lineage of *Polynucleobacter* (short curved rods, MCV of 0.054 μm^3 , Šimek et al. 2018). The cells were harvested at early stationary phase and mixed at a similar numerical ratio that yielded $\text{MCV} \pm \text{SD}$ of the mixture cells of $0.072 \pm 0.022 \mu\text{m}^3$, which matched well the typical MCV of bacteria in the fishponds ($0.075 \pm 0.029 \mu\text{m}^3$, median $0.066 \mu\text{m}^3$).

For grazing experiments, 300-mL samples were dispensed into rinsed 1-liter flasks and incubated at in situ temperature. The FLB tracers were added to constitute 5–19% of total bacteria, with the amounts added depending upon the season and water temperature. Notably, there is a very broad spectrum in ciliate species-specific uptake rates (e.g., Šimek et al. 1995; Stabell 1996). Thus in summer—a period of enhanced occurrence of peritrichous ciliates with very high uptake rates—we also ran incubations in parallel with very low FLB additions, constituting only 2–4% of total bacteria. We determined ciliate grazing rates in 5- and 10-min subsamples, and flagellate grazing rates in 30- or 45-min subsamples, with the longer times and greater tracer FLB amounts added during colder water periods. The incubations were terminated by the addition of 1% glutaraldehyde (final concentration). Duplicate subsamples of 2–6 mL (flagellates) or 4–30 mL (ciliates) were stained with DAPI, passed through 1- μm black filters (Osmonics), and inspected via epifluorescence microscopy to count both protistan groups (HNF at $\times 1000$ and ciliates at $\times 600$ magnification) as detailed in Šimek et al. (2014). All samples were inspected within 1–3 d after preservation. At least 100–200 ciliate and 200 HNF individuals (except for Rod during its clear-water phase) were inspected for FLB ingestion in

each sample. To estimate total protozoan grazing, we multiplied average bacterial uptake rates (based on tracer FLB uptake) of ciliates and HNF by their in situ abundances.

Composition of ciliate assemblages

The ciliate assemblage structure was evaluated by combining: (1) DAPI-stained samples in epifluorescence microscopy, (2) in some cases, live sample observation, and (3) quantitative protargol staining (for details, see Skibbe 1994 with modifications according to Pfister et al. 1999) on Lugol's fixed samples postfixed with Bouin's fluid. For protargol preparations, we filtered 5–20 mL of fixed samples on 0.8- μm pore-size Nitrocellulose filters (with counting grids, Sartorius). We identified and counted at least 200 individuals per sample. For more details on the above approaches and criteria used for grouping ciliates into different taxonomic categories, see Šimek et al. (1995) and Posch et al. (2015 and references therein). Because protargol staining was applied in parallel with fluorescence microscopy for ciliates, we determined most of the ciliates to the level of genus and, where possible, to species. Ciliates observed with fluorescence microscopy can be difficult to identify, so we used additional criteria, such as ciliate cell size, the position and size of nuclei, and the way in which prey were arranged in the food vacuoles (see also Šimek et al. 1995). An inspection of food vacuole contents provided additional information on the preferred prey and feeding modes of the ciliates, e.g., presence of FLB or phytoplankton cells based on their chlorophyll autofluorescence. The ciliates ingesting only picoplankton (prey $< 2 \mu\text{m}$, i.e., bacteria and picocyanobacteria) were ranked as bacterivorous, while ciliate taxa ingesting both pico- and nanoplankton (mostly algae 2–8 μm large) were considered to be omnivores. Between 5% and 15% of the ciliates, however, could not be identified by fluorescence microscopy, although their FLB uptake was quantified and thus included in the bulk ciliate bacterivory rate estimates. We based our identifications on the publications of Foissner and Berger (1996) and Foissner et al. (1994, 1999) and detailed references therein.

Due to mostly a low number of ciliate individuals of each taxon, which were inspected for grazing per sample, the grazing data of the same taxa (only unambiguously identified ciliates or their morphotypes) from the whole study period were pooled to calculate specific grazing rates at the genus, species, or morphotype levels.

Image capture of protistan cells with different prey items ingested

The images of protists with ingested prey were obtained with a motorized fluorescence microscope Nikon Eclipse 90i (Nikon, Tokyo, Japan) equipped with a monochromatic digital camera Andor Clara (Andor Technology, Belfast, UK), both controlled by the software NIS-Elements 4.12 (Laboratory Imaging). This software was also used for image processing; procedures were mostly automated using built-in system

functions. For each protistan cell, we captured a z-stack of multicolor images, each containing three color channels showing (1) DAPI fluorescence (bacteria, nuclei of protists and algae), (2) chlorophyll autofluorescence (algae and cyanobacteria), and (3) 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) fluorescence (FLB); each z-stack was stored in a single file in the ND2 format used by the NIS-Elements system. We typically took 10–20 images (focal planes) per z-stack in a 0.5- μm step, depending on the diameter of a protistan cell. We used specific microscope optical filter settings for DAPI (excitation/emission: 360–370 nm/> 420 nm), chlorophyll (510–550 nm/> 590 nm), and DTAF (448 nm/520–540 nm) fluorescence acquisition. To get enhanced 2D pictures, first, we used the proprietary EDF function of the NIS-Elements software that produces one focused 2D image by picking the in-focus regions from the z-stack images. The resulting image was then modified by adjusting input intensity range and the gamma parameter of each channel, merging the channels into the single RGB image, enhancing local contrast using the built-in function, and sharpening with the “unsharp mask” function. This image capture algorithm yielded, in one single composite picture, the information on the lateral position and shape of ciliate nuclei and the presence of prey items with distinct optical characteristics such as fluorochrome-labeled FLB, compared to algae and cyanobacteria with natural chlorophyll autofluorescence.

Zooplankton community composition and counting

Both the crustacean and rotifer samples were quantitatively processed using an open Sedgewick Rafter counting chamber mounted on a light microscope (Leica DM 3000 LED; McCauley 1984). Obtained densities were expressed as number of organisms per liter. For the purpose of this study, zooplankton were split into six categories: total Cladocera (including *Daphnia*), *Daphnia* separately, nauplii, copepodites, adults of Copepoda, and total Rotifera.

Basic chemical background data and chlorophyll *a* determination

Total organic carbon and dissolved organic carbon were determined with the Formacs^{HT} Analyzer (Skalar, Analytical B.V., The Netherlands). Total phosphorus, dissolved reactive phosphorus, and total nitrogen were determined via flow injection analysis with spectrophotometric assignment using a FIAstar 5012 (Foss, Denmark). Chlorophyll *a* (Chl *a*) was determined spectrophotometrically after the extraction of samples collected on GF/C filters as described elsewhere (Pechar 1987).

Meta-analysis of bulk ciliate bacterivory in systems of different trophic status

A meta-analysis of the relationship between Chl *a* concentrations (as a proxy of trophic status of lakes) and proportion of bacterial standing stock grazed daily by ciliates was conducted.

The studied ponds were analyzed together with available literature data from various lakes, yielding a data set consisting of eight subsets ($n = 46$): (1) 10 hypertrophic ponds from this study, plotted as mean values; (2) hypertrophic Furuike Pond (Japan)—mean values for spring, summer, and fall periods (data from Nakano et al. 1998); (3) hypertrophic Lake Vortsjärv (Estonia), a seasonal mean value (data from Zingel et al. 2007); (4) the highly eutrophic Sau Reservoir (Spain), mean values for lacustrine parts from two sampling campaigns conducted in April and July 1997 (for details, see Comerma et al. 2003); (5) the meso-eutrophic Římov reservoir (Czech republic)—three data sets were used: the mean value of 16 measurements for late summer in 1993 (Šimek et al. 1995), the mean value of 23 measurements over the spring bloom period, March–May 2009 (for details, see Šimek et al. 2014), and the mean value of 14 measurements, March–May 2018 (K. Šimek unpubl. data); (6) meso-eutrophic Oglethorpe Lake (Georgia, U.S.A., data from Sanders et al. 1989); (7) Lake Zurich—July 2010, five measurements on the longitudinal profile of the lower lake basin (K. Šimek and T. Posch unpubl. data); and (8) 16 oligo- to mesotrophic Norwegian lakes around Oslo (for details, see Stabell 1996).

Statistical analyses

Possible interactions between microbial food web components, phytoplankton biomass, and zooplankton were revealed by redundancy analysis (RDA). The forward selection procedure was used to select significant explanatory variables out of six zooplankton categories described above. The response variables were phytoplankton biomass, and total abundances of bacteria, picocyanobacteria, HNF, and ciliates, and the relative proportion (%) of the *Halteria/Pelagohalteria* group in total ciliate assemblage. The whole data set was transformed by $\log(x + 1)$ and centered by species. Each pond at a given sampling date was considered as an independent observation and permutation tests were performed in unrestricted design. Number of permutations was set to 9999. The RDA analysis was done using Canoco 5.10 (Ter Braak and Šmilauer 2012).

Results

Basic physical, chemical, and microbial characteristics of the fishponds

Altogether 33 analyses of microbiological and chemical parameters were conducted in 10 ponds over the period of April 2017–September 2017 (Fig. 1, Supporting Information Tables S1, S2). They indicated the hypertrophic status of ponds, reflected in fairly low water transparency (20–90 cm), high Chl *a* concentrations, and microbial abundances. For instance, based on 30 analyses from the bloom phase in the 10 ponds, the average (\pm SD) abundances of the heterotrophic microbes were $16.7 \pm 5.5 \times 10^6$ bacteria mL^{-1} , $8.6 \pm 7.0 \times 10^3$ HNF mL^{-1} , and 294 ± 199 ciliates mL^{-1} . The data from the pond Rod in April, May, and September differed from the rest of the data set as a temporal clear-water phase appeared, likely driven mainly by the

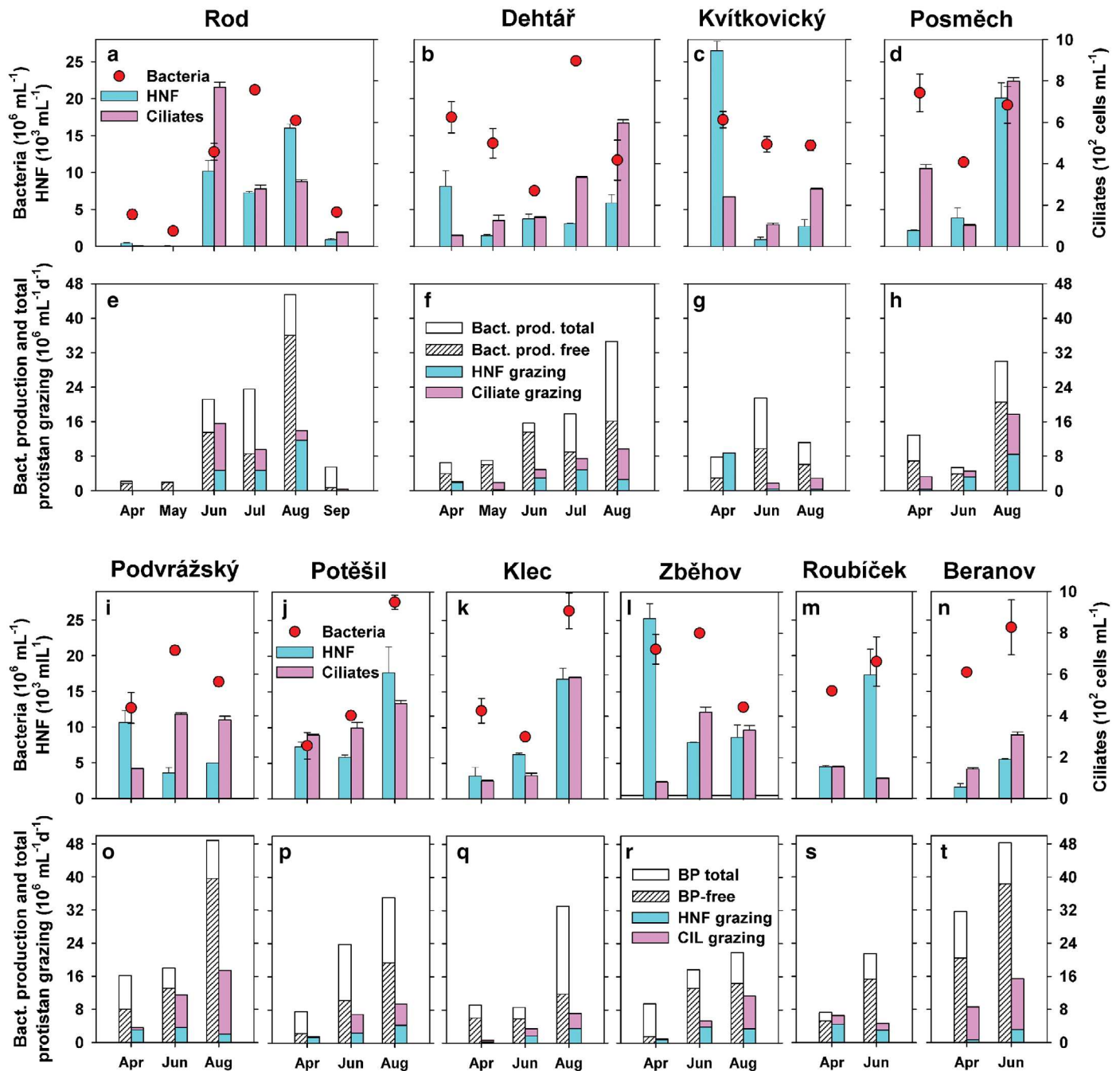


Fig. 1. Ponds Rod, Dehtář, Kvítkovický, Posměch, Podvrážský, Potěšil, Klec, Zběhov, Roubíček, and Beranov. Upper panels for the respective ponds (a–d and i–n): Total abundances of bacteria, HNF, and ciliates with error bars showing range of values of duplicates. Lower panels for the respective pond (e–h and o–t): Total BP and proportion of free bacteria (striped area in bars), compared to total aggregated protistan bacterivory subdivided into HNF and ciliate grazing shown in stacked bars.

occurrence of a different cladoceran assemblage in which mostly *Daphnia pulicaria* dominated (Supporting Information Fig. S1, Tables S3, S4). These two strikingly different plankton scenarios, differing in Chl *a* and abundances of microbes by more than one order of magnitude (Fig. 1, Supporting Information Tables S1, S2), were treated separately and the tests showed highly significant differences in Chl *a* and the microbial parameters (Mann–Whitney test, $p = 0.0004$ – 0.0007 ,

Mann–Whitney $U = 0$ – 1 , $n = 3$ [clear water], 30 [bloom phase], Table 1).

Bacterioplankton dynamics and mortality induced by protistan grazing

Overall, no common seasonal trends in bacterial dynamics were observed in the ponds (Fig. 1). However, common features of the bloom phase in the hypertrophic ponds were large

Table 1. Parameters of bulk protistan community bacterivory shown separately for ciliate and HNF communities and as aggregated total protistan bacterivory (mean \pm SD, range of values in parenthesis): (1) Cell-specific grazing rates of ciliates and HNF averaged over all 33 measurements conducted in the ponds (for details, see Supporting Information Table S1); (2) total grazing rate shown in absolute values; (3) relative values of grazing impact expressed as the percentage of bacterial standing stock grazed per day; (4) percentage of total BP grazed; and (5) percentage of BP grazed in the fraction of bacteria $< 1 \mu\text{m}$.

Parameter	Ciliates	Flagellates	Total protists
	Mean \pm SD (range of values)	Mean \pm SD (range of values)	Mean \pm SD (range of values)
(1) Cell-specific protistan grazing rate (bacteria cell ⁻¹ h ⁻¹)	523 \pm 502 (0–2328)	17.4 \pm 13.6 (2.3–65.5)	Not determined
(2) Total protistan bacterivory (10 ⁶ bacteria d ⁻¹)			
Clear water	0.10 \pm 0.16 (0–0.29)	0.05 \pm 0.04 (0.01–0.09)	0.15 \pm 0.20 (0.01–0.38)
Bloom phase	4.01 \pm 3.96 (0.39–15.4)	3.23 \pm 2.59 (0.23–11.7)	7.23 \pm 4.96 (0.72–17.7)
(3) % bacterial standing stock grazed per day			
Clear water	2.2 \pm 3.5 (0–6.3)	1.1 \pm 0.7 (0.4–1.9)	3.3 \pm 4.2 (0.4–8.2)
Bloom phase	24.9 \pm 23.6 (7.0–94.4)	20.3 \pm 15.4 (1.54–68.5)	45.2 \pm 30.4 (5.6–122)
(4) % total BP grazed			
Clear water	1.57 \pm 2.33 (0–4.25)	1.38 \pm 0.85 (0.45–2.02)	2.95 \pm 3.02 (0.45–6.94)
Bloom phase	20.3 \pm 11.9 (2.3–51.3)	18.0 \pm 16.8 (2.1–81.7)	38.3 \pm 24.9 (7.9–113)
(5) % BP grazed in the $< 1 \mu\text{m}$ fraction (free bacteria)			
Clear water	3.84 \pm 6.01 (0–10.7)	2.15 \pm 1.45 (0.49–3.16)	5.99 \pm 7.04 (0.49–13.9)
Bloom phase	37.1 \pm 24.1 (6.2–122)	33.8 \pm 33.1 (3.8–180)	71.1 \pm 51.9 (30.4–302)

Note: The mean values for the parameters (2)–(5) are presented separately for the clear-water phase (three samplings) in the Rod fishpond and the rest of 30 analyses during the bloom phase since they differed highly significantly (Mann–Whitney test, $p < 0.003$, Mann–Whitney $U = 1$, $n = 3$ [clear water], 30 [bloom phase]) between these two plankton successional phases.

numbers of diverse particles in the plankton, such as phytoplankton cells, their colonies, and suspended detrital or clay particles with attached bacteria (data not shown). This high variability within different ponds was also reflected in differences in the relative importance of BP of the free (the size fraction $< 1 \mu\text{m}$) and particle-attached bacteria (the size fraction $> 1 \mu\text{m}$, Fig. 1) that accounted, on average (\pm SD), for $56\% \pm 21\%$ and $44\% \pm 20\%$ of total BP, respectively. Average growth rates assessed via the thymidine uptake assay yielded the bacterioplankton doubling time of around 25 h (data not shown).

Considerable proportions of BP were consumed by ciliates and HNF (Fig. 1; Table 1). Notably, the contributions of ciliate and HNF bacterivory were similar, ciliates accounting for 56% compared to 44% attributable to HNF bacterivory. Bulk grazing rates (expressed both as the proportion of BP or daily removal rates of bacterial standing stocks) of HNF and ciliates were always significantly, approximately 10 times (Table 1), higher during the bloom phase than during the clear-water phase induced by zooplankton grazing. However, there was a large variability in the bulk grazing rates of these two protistan groups in the whole data set, and even temporarily within one particular pond (Fig. 1). This reflected both variable trends in protistan numbers and their community dynamics (Figs. 1–2) that were, moreover, apparently modulated by the trophic structure in the form of specific zooplankton compositions (Supporting Information Fig. S1, Tables S3, S4).

On the cell-specific basis, the HNF grazing rates ranged from 2 to 65 bacteria flagellate⁻¹ h⁻¹, with the average (\pm SD) of 17.4 ± 13.6 bacteria flagellate⁻¹ h⁻¹ (Table 1). The ciliate cell-specific bacterivory rates ranged from 0 to 2328 bacteria ciliate⁻¹ h⁻¹, with the overall average (\pm SD) of 523 ± 502 bacteria ciliate⁻¹ h⁻¹. The variability in ciliate grazing rates per cell mainly reflected ciliate assemblage compositions at different sampling dates and ponds, being primarily related to shifts between bacterivorous, omnivorous, or algivorous species with distinct abilities to ingest bacteria (Figs. 2–6, Table 2, and see below).

Ciliate assemblage composition and bacterivory

While the ciliate assemblage composition changed considerably in time and between different fishponds (Fig. 2), some general patterns in the representation of the higher ciliate taxa were apparent. In total, we determined the assemblage composition in 27 out of 33 samples; densities of mainly colonial cyanobacteria were so high in six samples that a quantitative evaluation of ciliates was not possible on silver impregnated slides. Based on all evaluated sampling sites and sampled seasons, the following rank order of ciliate subclasses was determined: Stichotrichia (27.6%), Prorodontida (25.5%), Scuticociliata (13.2%), Oligotrichia (12.4%), Choreotrichia (6.8%), Haptoria (6.7%), Peritrichia (4.0%), and Colpodea (2.9%). In ponds with a high proportion of Prorodontida, this group was composed entirely of typical phytoplankton feeders or flagellate hunters, such as *Balanion planctonicum* and *Urotricha*

spp. and, to a much lesser extent, by *Coleps* spp. (Supporting Information Table S5). In some cases, these algivores even exceeded 50% of total ciliates; however, their contribution to total ciliate bacterivory was quite negligible (Fig. 2).

Occasionally, ponds were numerically dominated by small omnivorous Oligotrichia, particularly of the genus *Rimostrombidium*, represented mainly by the species *R. humile*, *R. hyalinum*, and *R. brachykinetum* (Supporting Information Table S5). Ciliate

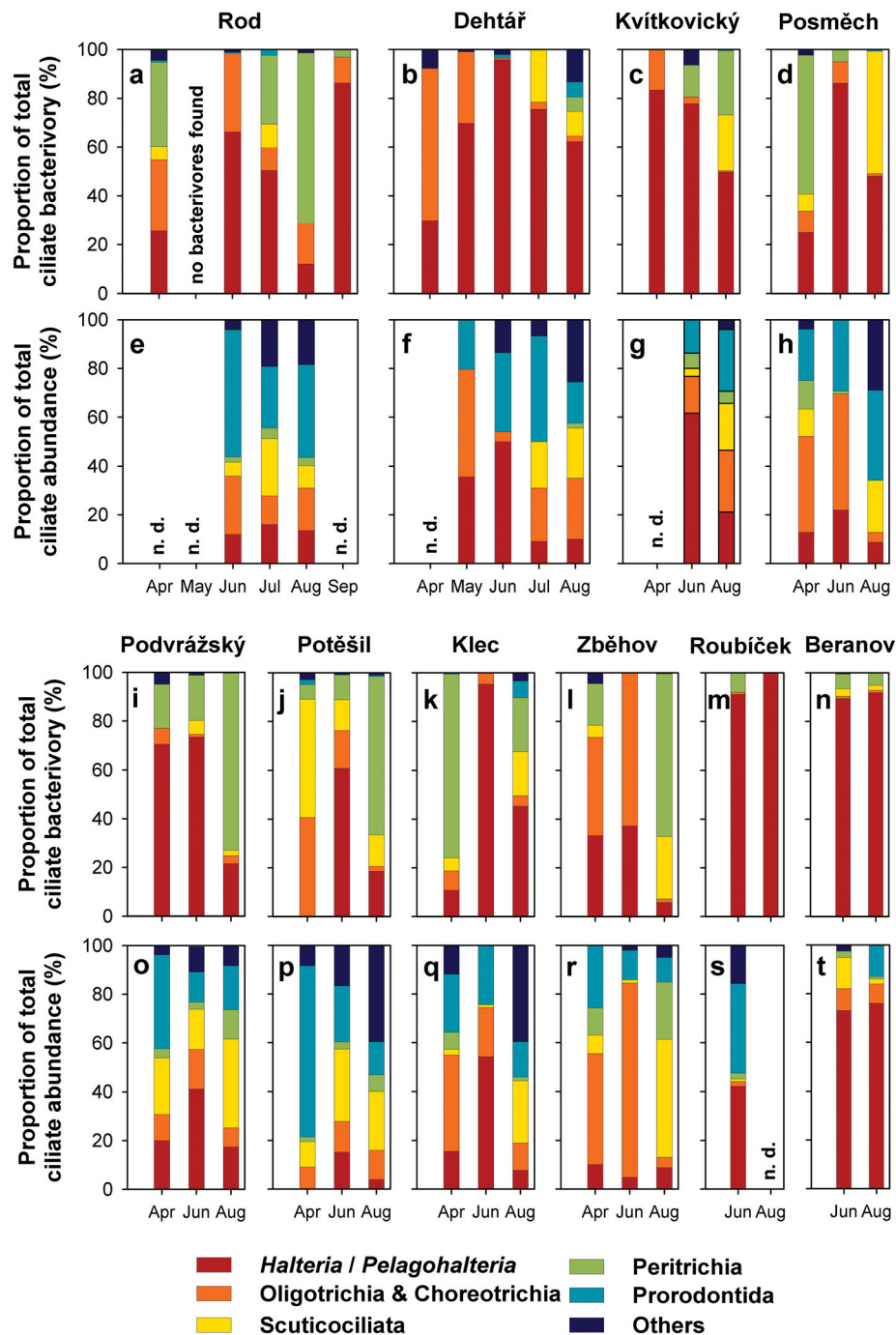


Fig. 2. Upper panels (a–d, ponds Rod, Dehtář, Kvítkovický, and Posměch; i–n, ponds Podvrážský, Potěšil, Klec, Zběhov, Roubíček, and Beranov): Relative contributions of six major taxonomic groups (*Halteria*/*Pelagohalteria* spp., *Oligotrichia* and *Choreotrichia*, *Scuticociliata*, *Peritrichia*, *Prorodontida*, and “others”) to total ciliate bacterivory based on FLB uptake assays. Microphotographs of the major bacterivorous morphospecies with ingested FLB and with chlorophyll-containing phytoplankton cells are presented in Figs. 3–5. Lower panels (e–h and o–t) show assemblage composition of these six ciliate groups (based on protargol staining) in the ponds, expressed as relative contributions to total ciliate abundance. For details of the ciliate assemblage composition in each pond, see Supporting Information Table S5.

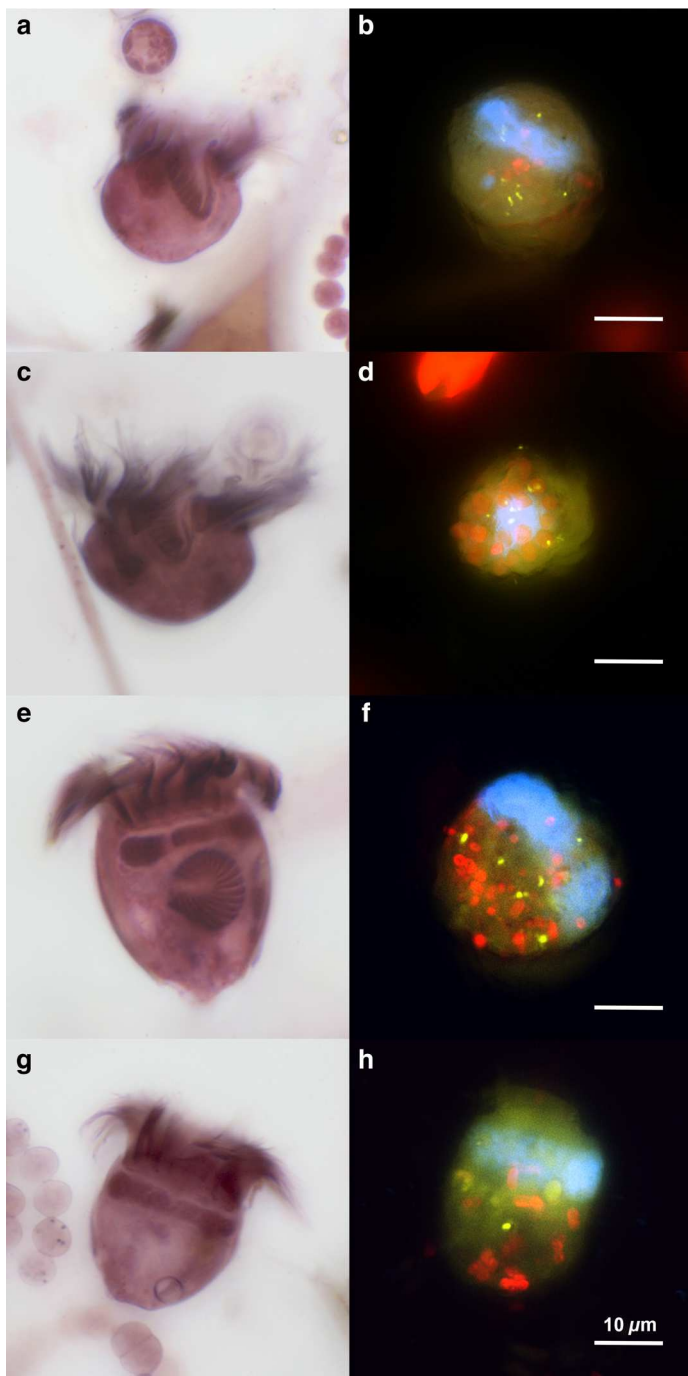


Fig. 3. Examples of major bacterivorous ciliate morphospecies in ponds: Stichotrichia and Oligotrichia. (a–d) *Halteria/Pelagohalteria*, a zoochlorellae-bearing individual shown in (d); (e–h) two morphotypes of the *Rimostrombidium* complex with remarkable uptake of small algae along with FLB. Left side (a, c, e, g)—a protargol-stained cell, right side (b, d, f, h)—examples of the same species in fluorescence microscopy based on composite overlay of 10–20 images taken in three wavelengths (blue: DAPI-excitation visualizing mainly nuclei of ciliates, yellow: DTAF-excitation visualizing FLB uptake, red: Chlorophyll excitation visualizing natural phytoplankton cells ingested in food vacuoles). The common presence of FLB and red phytoplankton cells in food vacuoles documents the ciliate omnivory. The scale bar shows length of 10 μm .

assemblages of ponds were often numerically dominated by members of Stichotrichia, especially by the genera *Halteria* and *Pelagohalteria*. Interestingly, in several cases with particularly high cladoceran abundance (800–1380 ind L^{-1} , dominated mainly by

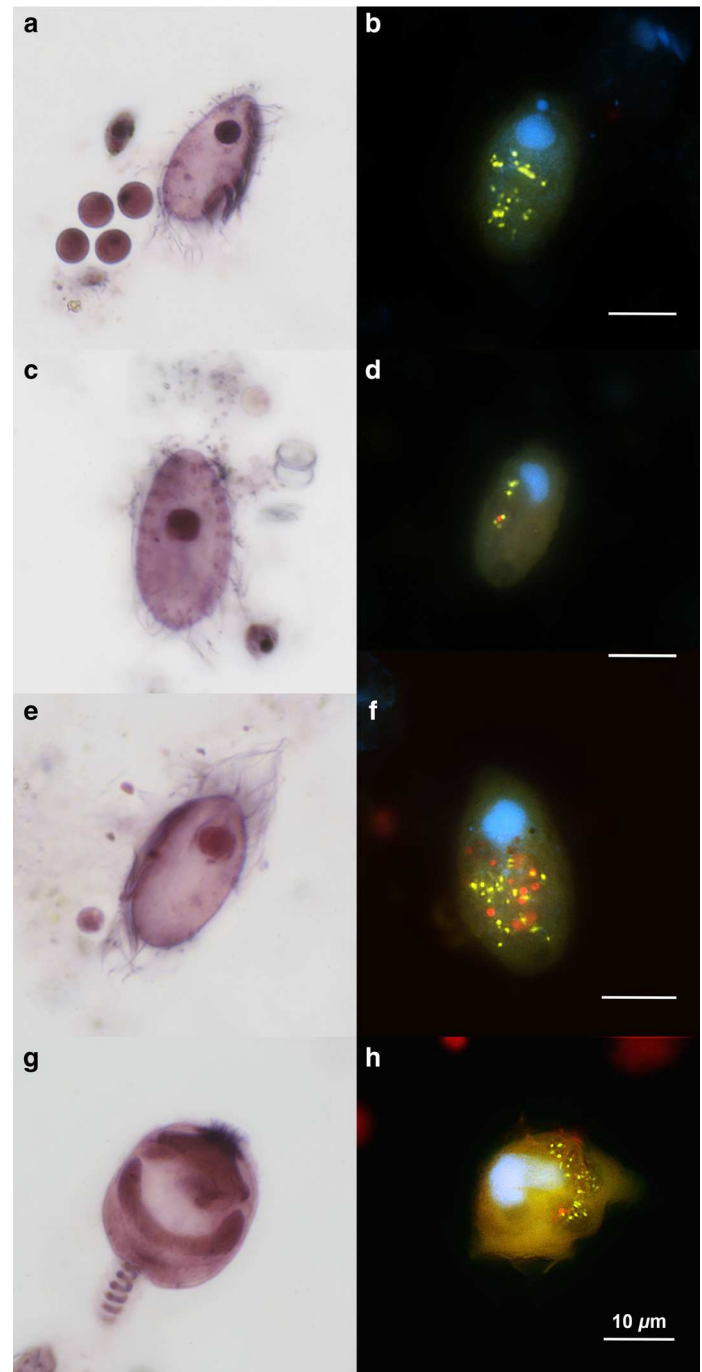


Fig. 4. Examples of major bacterivorous ciliate morphospecies in ponds: Scuticociliata and Peritrichia. (a–f) Three morphospecies of *Cyclidium* spp.; (a, b), *C. glaucoma*; (c, d), an unidentified *Cyclidium*-like scuticociliate; (e, f) *C. heptatrichium*; (g, h) a small *Vorticella* sp. For more details, see Fig. 3 caption.

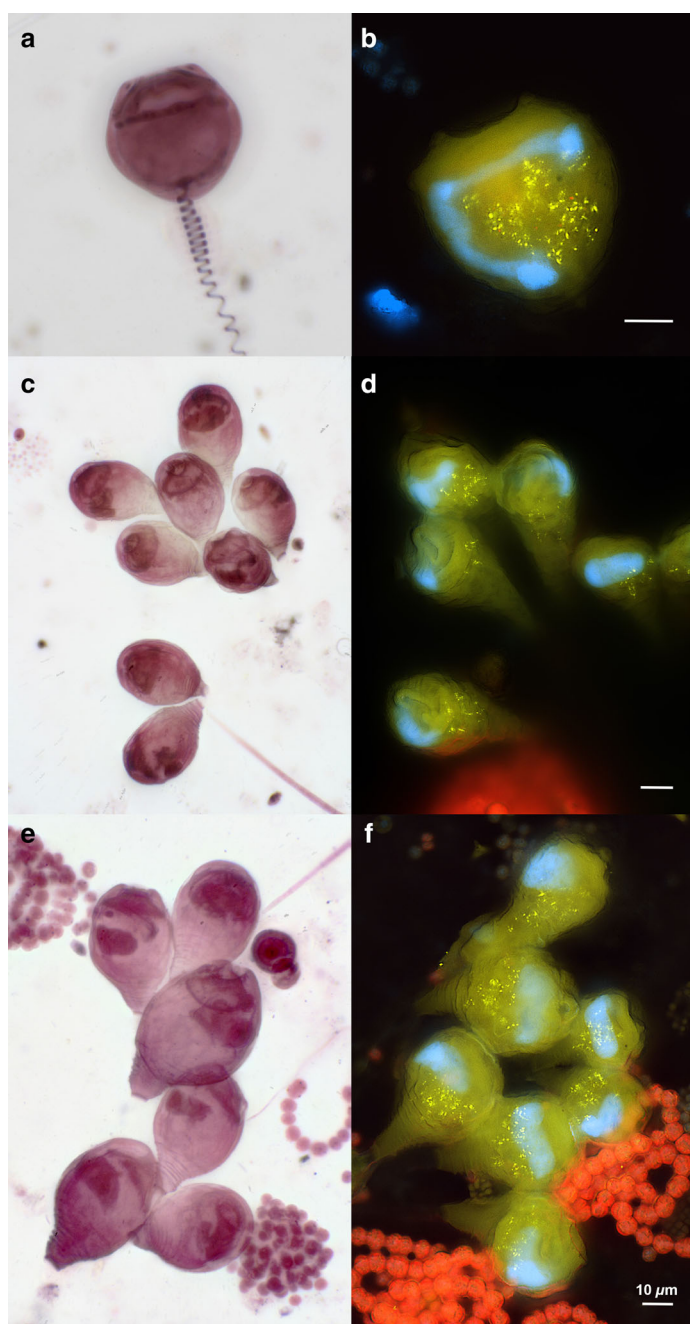


Fig. 5. Examples of major bacterivorous ciliate morphospecies in ponds: Peritrichia. (**a**, **b**) *P. natans*; (**c**–**f**) a colony of *E. procumbens*. For more details, see the Fig. 3 caption.

Bosmina longirostris, see Supporting Information Table S4), the ciliate assemblage was almost entirely dominated by the genus *Halteria* (see Beranov and Roubíček in Fig. 2, cf. Supporting Information Fig. S1).

In terms of bulk ciliate bacterivory, the most important ciliate bacterivores in order of their importance were (Figs. 2–6): (1) Stichotrichia, primarily the omnivorous *Halteria* and *Pelagohalteria* spp., (2) in some cases, Oligotrichia (namely

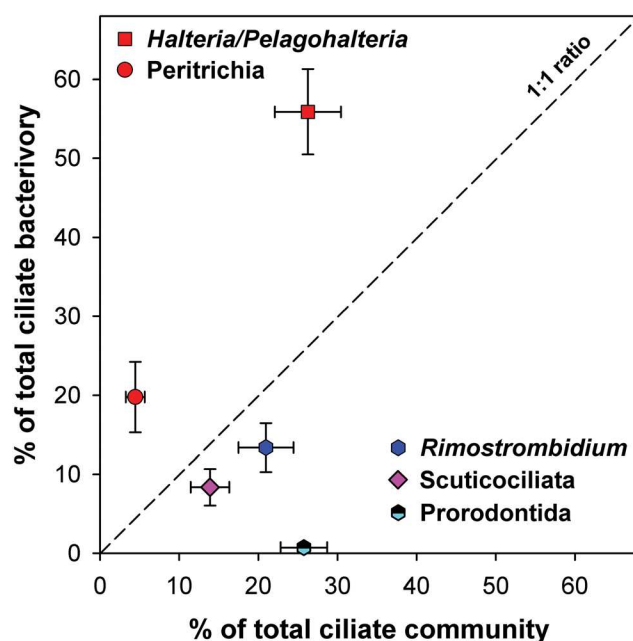


Fig. 6. Average contributions of five major ciliate groups ingesting bacteria to total ciliate bacterivory, based on all samples from 10 ponds ($n = 33$). Dashed line indicates the 1:1 ratio, i.e., the equal contribution of a ciliate group to total ciliate bacterivory as well as to total ciliate assemblage composition. The groups with over-proportional contributions to bulk ciliate bacterivory, *Halteria/Pelagohalteria* and Peritrichia, are plotted as red symbols. The groups contributing disproportionately less to the bulk ciliate bacterivory, Scuticociliata, *Rimostrombidium*, and Prorodontida are plotted below the 1:1 line ratio. Error bars depict bidirectional values of standard errors of the means.

Rimostrombidium spp., see Fig. 2) also contributed markedly. These taxonomic groups were followed by (3) highly efficient picoplankton grazers detected within Peritrichia (namely genera *Vorticella* and *Epistylis*, Table 2), (4) Scuticociliata (mainly genus *Cyclidium*, see Fig. 4) and rather negligible role of Prorodontida (only *Coleps* spp. was detected as a poor bacterivore, Table 2). However, at the genus level, *Halteria/Pelagohalteria* spp. were clearly the most important bacterivorous genera in the ponds, accounting, on average, for 57% of total ciliate grazing (Fig. 6) and for 30% of total aggregated (HNF plus ciliate) bacterivory.

A general overview on the contribution of major taxa to total ciliate bacterivory is depicted in Fig. 6. It clearly documents the overproportional contributions of the *Halteria/Pelagohalteria* group and of Peritrichia (red symbols in Fig. 6) to total ciliate bacterivory rates compared to their proportions in the total assemblage. An opposite trend with slightly under-proportional contributions to the bulk ciliate bacterivory is obvious for the groups Scuticociliata and *Rimostrombidium* spp., while contributions for Prorodontida were negligible.

To unveil more general trends in the role of ciliate bacterivory along a gradient from oligo- to hypertrophic lakes, we conducted a meta-analysis of available literature data on

Table 2. Cell-specific grazing rates of the 10 most important planktonic ciliate taxa or morphotypes detected as bacterivores in ponds, the data are shown as mean \pm SD, median, and range of values.

Taxonomic group	Cell-specific grazing rates (bacteria ciliate ⁻¹ h ⁻¹)			
	Mean \pm SD	Median	Range	n
Spirotrichea—Stichotrichia				
<i>Halteria/Pelagohalteria</i>	1,359 \pm 1,089	1,089	145–5,866	631
Spirotrichea—Oligotrichia				
<i>Rimostrombidium</i> spp.	304 \pm 354	210	0–1,875	223
Scuticociliata				
<i>Cyclidium glaucoma</i>	558 \pm 407	480	55–2,179	201
An unidentified <i>Cyclidium</i> -like scuticociliate (16 \times 12 μ m)	775 \pm 327	662	311–1,570	45
<i>Cyclidium heptatrichum</i> (ca. 21 \times 14 μ m)	648 \pm 182	662	265–1,058	41
<i>Cinetochilum margaritaceum</i>	47 \pm 104	0	0–527	85
Prorodontida				
<i>Coleps</i> spp.	190 \pm 267	0	0–793	40
Peritrichia				
<i>Vorticella aquadulcis</i> complex (ca. 22 \times 18 μ m)	2,951 \pm 1,362	2,976	406–6,008	70
<i>Pelagovorticella natans</i> (ca. 40 \times 38 μ m)	10,512 \pm 2,913	10,552	4,059–18,940	53
<i>Epistylis procumbens</i> (ca. 51 \times 29 μ m)	11,261 \pm 3,410	11,229	6,088–20,293	41

bulk ciliate bacterivory (as percentage of bacterial standing stock grazed per day) and data on a Chl *a* concentration as proxy for the habitat's trophic status (Fig. 7). The statistically significant positive trend line clearly indicates an increasing role of ciliate bacterivory along the trophic gradient toward hypertrophic habitats (red symbols in Fig. 7) since the variability in Chl *a* explained 72.6% of the variability in aggregated ciliate bacterivory rates.

Ciliate species-specific uptake and clearance rates

Cell-specific bacterivory rates of the ciliate taxa are reported in Table 2. While Peritrichia were never as abundant as the algivorous Prorodontida, omnivorous *Halteria/Pelagohalteria* or *Rimostrombidium* spp., already moderate concentrations of Peritrichia frequently led to a prominent role in ciliate bacterivory (Fig. 2). This is related to the fact that species such as *Epistylis procumbens* (Fig. 5c–f) and the larger species *Pelagovorticella natans* (Fig. 5a,b) showed the highest cell-specific uptake rates of 11,261 and 10,512 bacteria ciliate⁻¹ h⁻¹ and clearance rates of 733 and 687 nL ciliate⁻¹ h⁻¹, respectively (Table 2, Supporting Information - Table S6). Also, a small species of *Vorticella* sp. (Fig. 4g,h) displayed high grazing rates, almost 3000 bacteria ciliate⁻¹ h⁻¹, accompanied, moreover, by the highest measured volume-specific clearance rate (VSCR, 6.27×10^4 nL h⁻¹; Table 2, Supporting Information Table S6).

Overall, the most important bacterivores, the group of *Halteria/Pelagohalteria* (Figs. 2, 6), displayed average uptake rates of 1359 bacteria ciliate⁻¹ h⁻¹. Similarly sized species of the genus *Rimostrombidium* showed approximately four times lower uptake, clearance, and VSCR rates and we also detected more often small algae in food vacuoles in parallel with

ingested tracer FLB (see Fig. 3e–h, Table 2, Supporting Information Table S6). Moderate uptake rates of 558 (*Cyclidium glaucoma*) and 775 bacteria ciliate⁻¹ h⁻¹ (an unidentified

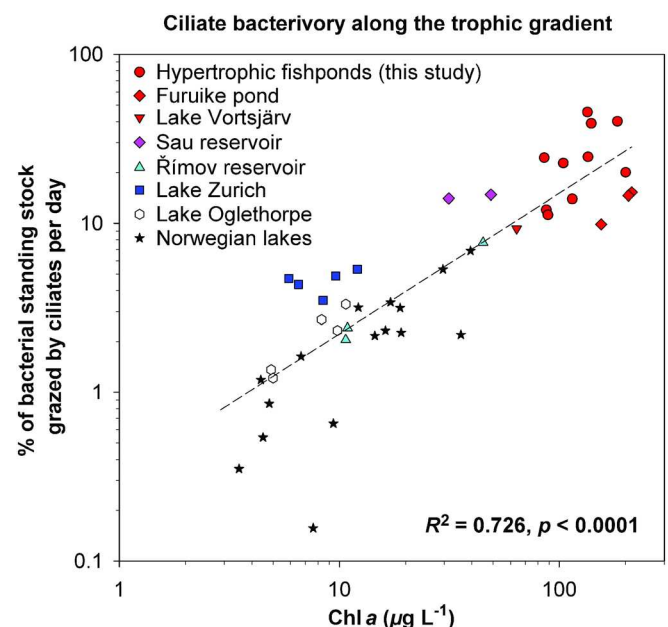


Fig. 7. The meta-analysis of the relationship between Chl *a* concentrations (as a proxy of trophic status of lakes) and proportion of bacterial standing stock grazed daily by ciliates; shown as log-log linear regression (a dashed line). The studied systems were analyzed together with the available literature data from various lakes along the trophic gradient from oligotrophy to extreme hypertrophy ($n = 46$). For details of the habitats and corresponding references, see “Materials and methods” section. Note that all hypertrophic systems are plotted as red symbols.

scuticociliate, Table 2) were found for typical small species of bacterivorous scuticociliates. Similar bacterial uptake rates were also detected for the slightly bigger scuticociliate *Cyclidium heptatrichium*, in which very high numbers of small autotrophs were observed as well (Fig. 4e,f). Very low bacterivory rates were detected in *Cinetochilum margaritaceum* (Scuticociliata) and *Coleps* spp. (Prorodontida). The VSCR values (Supporting Information Table S6) indicated that *Halteria/Pelagohalteria* spp., small Scuticociliata, and all Peritrichia were the best-adapted species to rapidly crop planktonic bacteria. However, primarily omnivorous genera with a slightly different feeding niche, i.e., fine filter-feeding *Halteria* and *Pelagohalteria*, and nanoplankton feeding *Rimostrombidium* spp., contributed significantly more (on average 71%, see Fig. 8) to total ciliate bacterivory than typical bacterivorous Peritrichia and Scuticociliata (on average only 26%).

Relationships within planktonic food webs

An overview of prominent species of rotifers, cladocerans, and copepods found in the ponds is presented in Supporting Information Table S4. To unveil possible significant relationships between major zooplankton groups and microbial food web components, we conducted a RDA analysis (Fig. 9). Total rotiferan and cladoceran abundances together with copepodites were the explanatory variables, which explained 34% of the variability in

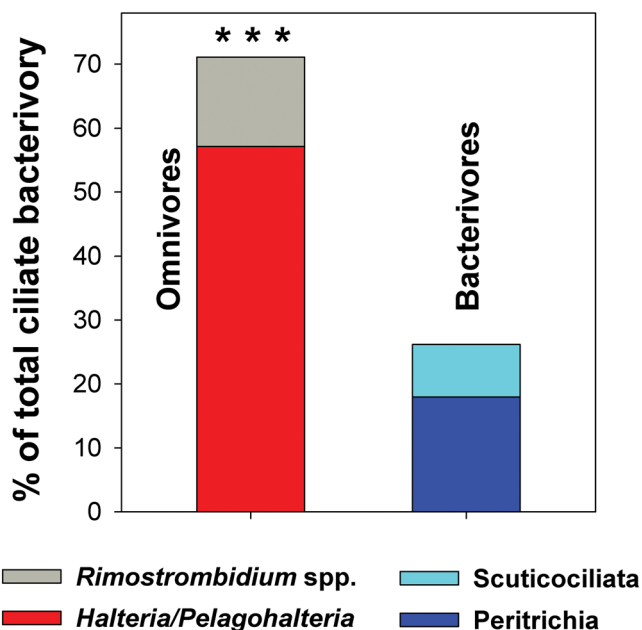


Fig. 8. Average contributions of four major ciliate groups ingesting bacteria to total ciliate bacterivory, based on all samples from 10 ponds. Fine-filter feeding genera *Halteria/Pelagohalteria* and nanoplankton feeding *Rimostrombidium* are omnivores, i.e., ingesting also algae 2–8 μm large, while Scuticociliata and Peritrichia are primarily specialized picoplankton grazer (prey < 2 μm , see also Figs. 3–5). The omnivorous taxa contribute significantly more (Wilcoxon test, $n = 32$, $\text{df} = 31$, $*** p < 0.001$) to total ciliate bacterivory than the taxa considered as typical bacterivores.

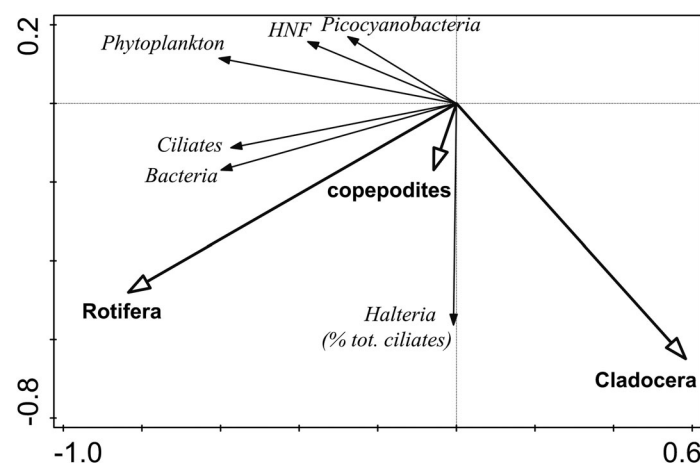


Fig. 9. Redundancy analysis based on data from all ponds demonstrating the influence of major zooplankton groups selected by the forward selection procedure (thick arrows, explanatory variables—Cladocera, Rotifera, and copepodites in bold) on phytoplankton biomass and major groups of microbes (thin solid arrows, response variables—group names in italics): Total bacteria, picocyanobacteria, HNF, and ciliate abundances, and the relative proportion of the *Halteria/Pelagohalteria* group in total ciliate community. Note that the relative proportion of the *Halteria/Pelagohalteria* group was the only ciliate group in the total ciliate assemblage with a unique response type reflected in positive correlation with Cladocera (*Halteria*, percentage of total ciliates; for more details, see the text). The model explains 34% of the variability in the data (Rotifera accounts for 18.3%, total Cladocera 9.2%, and copepodites 6.5% of variability). The copepodites consist of all copepodite stages of both the cyclopoid and calanoid copepods.

phytoplankton biomass and microbial food web components, representing the response variables in the analysis. Cladocerans negatively correlated mainly with picocyanobacteria, HNF, and phytoplankton biomass, and less significantly also with total ciliate and bacterial abundances. Only the relative proportions (not absolute numbers) of *Halteria/Pelagohalteria* in the ciliate assemblage positively correlated with cladoceran abundance; the trends were obvious, e.g., in ponds Dehtář, Beranov, and Roubíček (Figs. 1–2, Supporting Information Fig. S1). In contrast, the abundance of rotifers, dominated by fine-filter feeding genera such as *Keratella* and *Brachionus* (see Supporting Information Table S4), positively correlated with both bacterial and ciliate densities.

Discussion

We found that ciliates are frequently major protistan bacterivores in hypertrophic fishponds. Our studied ponds and two other shallow hypertrophic lakes (Nakano et al. 1998, Zingel et al. 2007, red symbols in Fig. 7) formed a well-separated cluster with extreme levels of both ciliate bacterivory and Chl *a* concentrations. The significant positive relationship (Fig. 7) strongly supports the hypothesis of an increasing role of ciliate bacterivory along the trophic gradient toward hypertrophic habitats.

Furthermore, we found that the major ciliate bacterivores are, in fact, omnivores with a substantial contribution of small

autotrophs in their diet (Figs. 3–6). Thus not specialized bacterivores, but omnivores, mainly fine-filter feeding *Halteria/Pelagohalteria* and to a lesser extent also *Rimostrombidium* spp. (Müller and Schlegel 1999), were most important bacterivores in the ponds. These three taxa from Stichotrichia and Oligotrichia accounted, on average, for 71% of total ciliate bacterivory (Fig. 8) and for 40% of total aggregated (HNF plus ciliate) protistan bacterivory. In contrast, peritrichs and scuticociliates, considered as specialized picoplankton grazers (e.g., Beaver and Crisman 1989; Foissner and Berger 1996), contributed significantly less (26%) to the total ciliate bacterivory. This supports the hypothesis that the omnivorous ciliates are major bacterivores in hypertrophic habitats.

Bacterioplankton mortality

For the bloom phase (30 data sets), the aggregate total protistan bacterivory represented approximately 38% and 71% of total and free BP, respectively. However, our BP estimates should be interpreted with some caution since we did not use an empirically derived conversion factor of thymidine incorporation to numbers of bacterial cells produced, but the overall mean value derived from various aquatic ecosystems studied (Ducklow and Carlson 1992). Our estimates indicated roughly one bacterioplankton population doubling per day, while total protistan bacterivory removed only around 45% of bacterial standing stock daily with a greater contribution of ciliates than HNF (Table 1).

In contrast, a temporally low fish stock contributed to the clear-water phase in Rod, where most planktonic organisms were efficiently cropped by zooplankton (Fig. 1), with the dominance of large-bodied *D. pulicaria* (and partly also *D. magna*), as the keystone species in the trophic structure (Jürgens et al. 1999; Sommer et al. 2012). This resulted in approximately five times lower bacterial abundances and BP rates in Rod. Thus, relatively low abundances of large-bodied daphnids controlled plankton dynamics including bacterial and phytoplankton productions, with particularly dramatic decreases in HNF and ciliate densities. This was reflected also by a significant negative effect of Cladocera on protistan abundances (Fig. 9). Consequently, the heavily grazed HNF and ciliate populations controlled only about 3% of total BP during the clear-water phases in Rod (Table 1).

Our data suggest that approximately 55% and 29% of the total and free bacterial mortality, respectively, were due to sources other than protistan bacterivory, such as grazing by larger zooplankton, namely by rotifers and Cladocera, or by viral infection. In accordance, many species of *Daphnia* (e.g., *D. pulicaria* and *D. longispina*) and rotifers, found in the ponds (genera *Keratella*, *Brachionus*), are typical bacterivorous or fine-filter feeding species which can efficiently feed on both free and particle-attached bacteria (Arndt 1993; Jürgens 1994; Jürgens and Jeppesen 2000). An important role of bacteria as a food source for rotifers and ciliates was also indicated by the statistical analysis (Fig. 9).

Ciliate composition, bacterivory, and species-specific uptake rates

We identified major ciliate bacterivores, either to the genus, or if possible to the species level. In some cases, we could not unambiguously couple the species identification from protargol preparations to individuals observed via epifluorescence microscopy and so we defined only a morphospecies group such as *Halteria/Pelagohalteria*, or *Rimostrombidium* spp. However, even this lower taxonomic resolution yielded important insights into the role of different ciliate taxa as bacterivores. Two different levels of such an evaluation should be distinguished: (1) the contribution of the ciliate taxon to total ciliate bacterivory and (2) the cell-specific uptake rates of the taxa. Regarding the overall contribution to bacterial mortality in all ponds, the most important bacterivores in order of their importance were (Figs. 2–6): (1) the group *Halteria/Pelagohalteria*, (2) Peritrichs (*Vorticella*, *Epistylis*), (3) *Rimostrombidium* spp., and (4) Scuticociliates (*Cyclidium* spp.).

Maximum ciliate numbers in the ponds, up to 800 cells mL⁻¹, were comparable to values found in wastewater treatment plants with moderate loads (Foissner and Berger 1996; Madoni 2011). However, the assemblage composition is quite distinct, mainly due to the presence of small algae and picocyanobacteria that are easily ingested by ciliates. Practically all the small and abundant “bacterivorous” ciliates with uptake rates > 500 bacteria ciliate⁻¹ h⁻¹, detected in the ponds (mainly *Halteria/Pelagohalteria*, *Rimostrombidium*, and *C. heptatrichium*), had a substantial contribution of algae in their diet. The most important ciliate bacterivores were typical suspension-filter feeders that obviously did not select for or against particular small prey items. Since we used FLB prepared from typical freshwater bacteria with small cell volumes, mimicking quite well the MCV of bacterioplankton in the fishponds, we assume that our bacterivory rate estimates are quite realistic (cf. Ståbel 1996; Nakano et al. 1998; Šimek et al. 2000; Comerma et al. 2003).

Species-specific differences in bacterivory rates (Table 2) likely reflect typical functional response of the ciliates regarding preferred food size. For instance, *Halteria* cf. *grandinella* has an optimal prey size of around 2.8 µm (Jürgens and Šimek 2000), yet being very efficient picoplankton grazer. Our results confirm that the unique ecological role and competitive success of the group *Halteria/Pelagohalteria* holds even under extreme hypertrophic conditions (cf. Nakano et al. 1998; Šimek et al. 2000). This group frequently dominated, especially during the presence of high numbers of *Daphnia* and *B. longirostris*, but also of copepods and rotifers, which might be related to specific escape reactions from zooplankton grazers (Tamar 1979; Gilbert 1994; Šimek et al. 2000). Given the abundant large algae and cyanobacterial flocks, *Halteria/Pelagohalteria* thus may outcompete other omnivorous ciliates from the group Oligotrichia (namely *Rimostrombidium* spp.). This finding is also supported by the significantly positive correlation between Cladocera abundance and the relative proportion of *Halteria/Pelagohalteria* in the ciliate assemblages (Fig. 9). In contrast, the increasing numbers of Cladocera had

significantly negative effects on total ciliate numbers as well as on abundances of all other ciliate groups presented in Fig. 2. This finding is particularly interesting in the naturally highly structured and “refuge-rich” hypertrophic plankton environment for small ciliates, since, e.g., cyclopoids were reported to ingest *Halteria* sp. at high rates under simplified experimental conditions (Wickham 1995).

The *Rimostrombidium* group is of comparable cell size as *Halteria/Pelagohalteria*; however, it likely does not possess an efficient escape reaction. The *Rimostrombidium* group showed a marked contribution of 4–8 μm large algae in their diet (see also Müller and Schlegel 1999; Posch et al. 2015), but had significantly lower bacterivory rates compared to *Halteria/Pelagohalteria* group.

The position of Peritrichia as the second most important ciliate bacterivores (Figs. 6, 8) is attributable rather to their high species-specific uptake rates than to their relative abundance. Such high cell-specific uptake rates are typical for fine-suspension feeding peritrichous ciliates in sludge systems or their sessile forms attached to zooplankton or to cyanobacterial colonies (Carrias et al. 1996; Foissner and Berger 1996; Madoni 2011). Notably, the uptake rates of *E. procumbens* and *P. natans* (Table 2) are likely the highest ever measured in situ species-specific bacterivory rates in pelagic environments (cf. Šimek et al. 1995; Stabell 1996; Bickel et al. 2012). All three important peritrichous ciliates (Figs. 4–5) were found to be attached on cyanobacterial colonies, namely on *Microcystis aeruginosa* or alternatively on *Staurastrum* sp., while they were almost never attached on zooplankton.

The fishponds hosted four distinguishable scuticociliates considered as primarily bacterivorous (Table 2, cf. Foissner et al. 1994). While *C. margaritaceum* is generally a low-efficiency bacterial grazer (Šimek et al. 1996; Nakano et al. 1998), the three morphospecies of the genus *Cyclidium* turned out to be important bacterial consumers. Only two smaller species of *Cyclidium* were the true picoplankton feeders that ingested only cells smaller than 2 μm (bacteria and picocyanobacteria), while in food vacuoles of *C. heptatrichium* (size 20–22 μm), we found a significant contribution of small algae (up to 4 μm). This finding ranks this species as an omnivore rather than the assumed bacterivore (cf. Foissner et al. 1994).

Broader ecological implications of ciliate omnivory in hypertrophic fish ponds

Compared to oligo- and meso-eutrophic lakes (e.g., Müller et al. 1991; Šimek et al. 2000; Posch et al. 2015), the ponds hosted markedly higher absolute numbers as well as relative proportions of omnivorous Stichotrichia and Oligotrichia (*Halteria/Pelagohalteria*, *Rimostrombidium*) and of bacterivorous Peritrichia (*Epistylis*, *Vorticella*, *Carchesium*) with very high bacterivory rates (see also Bickel et al. 2012). Peritrichia also disproportionately contributed to total ciliate bacterivory compared to their average abundance contributions to the total ciliate assemblage.

We found particularly high ciliate numbers and proportions of omnivorous taxa in ponds with a rather low C:P ratio (Posměch, Podvrážský, and Zbřehov; data not shown). This implies that these omnivores, as well as typical algivorous ciliates such as *B. planctonicum*, *Urotricha*, and *Askenasia* spp., might significantly contribute to the consumer-driven nutrient dynamics with rapid phosphorus recycling (Elser and Urabe 1999; Atkinson et al. 2017) in these ponds.

The relative contributions of different ciliate taxa in the different plankton assemblages, the direct microscopical evidence of the food ingested (Figs. 3–5), along with shifts in ciliate abundance and bacterivory along the trophic gradient, clearly points to competitive advantages of omnivory in hypertrophic systems. On the other hand, the specialized bacterivorous ciliates such as tiny scuticociliates (*Cyclidium*, *Uronema*, Fenchel 1980; Foissner et al. 1994) contributed less to the bulk ciliate bacterivory in the ponds.

The plentiful small algae and picocyanobacteria, large bacterial biomass with the presence of small (2–8 μm) bacterial flocks in the plankton likely underlie the success of omnivorous feeders (Diehl 2003; Weisse 2017). This makes these ciliate taxa far more important in the overall carbon flow to the grazer food chain than is the role of generally smaller HNF, with their vast majority being rather specialized picoplankton grazers (Arndt et al. 2000; Boenigk and Arndt 2002). For instance, we calculated that the phytoplankton size fraction from 0.5 to about 8 μm , available to the majority of small omnivorous ciliates, represented approximately a four times larger carbon pool (J. Nedoma unpubl. data) than the total carbon biomass of bacterioplankton. This finding implies that small omnivorous ciliates represent one of the principal carbon pathways to the grazer food chain in hypertrophic systems that can markedly reduce the number of trophic steps, which results in more direct carbon flow from microbes to zooplankton and to fish.

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Conflict of Interest

None declared.

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